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AN EXAMINATION OF THE METHODS FOR THE MICROCHEMICAL DETECTION OF PHOSPHORUS COMPOUNDS OTHER THAN PHOSPHATES IN THE TISSUES OF ANIMALS AND PLANTS.

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The microchemical reaction for the detection of phosphorus in the tissues of animals and plants introduced in 1898 by Macallum ('98) is a modification of that devised in 1893 by Lilienfeld and Monti ('93). These investigators attempted to demonstrate the distribution of phosphorus in tissues by subjecting the latter for some time to the action of a solution of ammonium molybdate in nitric acid, after which they were treated with a solution of pyrogallic acid. The nitric molybdate reagent was supposed to liberate the phosphorus from its organic combinations, to convert it into orthophosphoric acid, and finally to precipitate the latter as the yellow phosphomolybdate of ammonium. The further treatment of the tissues with pyrogallic acid had for its object the reduction of the ammonium phosphomolybdate to a lower oxide of molybdenum, which, according to Lilienfeld and Monti had a brown or black color. In this way the pale yellow precipitate containing the phosphorus was converted into a dark-colored precipitate which could be easily studied under the microscope.

The importance of a microchemical reaction which would enable us to determine accurately the distribution of the compounds of phosphorus not only in the tissues but also in the parts of the cells of the tissues can hardly be overestimated. It is therefore

not surprising that the results of Lilienfeld and Monti have been subjected to careful experimental examination by a number of other investigators.

Raciborski ('93) in 1893, in his review of Lilienfeld and Monti's article, showed that the reaction of ammonium phosphomolybdate with pyrogallic acid resulted in the production of a green compound, while ammonium molybdate gave by reduction with the same reagent a brown compound. He concluded that the brown reaction of Lilienfeld and Monti was due to ammonium molybdate mechanically imbibed by the section, and not to ammonium phosphomolybdate.

Heine ('96), also, showed that phosphorus-free histone, prepared from the thymus, formed with the nitric-molybdate reagent compounds from which the ammonium molybdate could not be removed by washing in water, and in which it could be detected by the use of reducing compounds. For this purpose he employed stannous chloride.

Macallum confirmed Raciborski's observations as to the color compounds produced by the reduction of ammonium phosphomolybdate and ammonium molybdate respectively, and showed that ammonium molybdate could not be removed from tissues which had been treated with the nitric-molybdate reagent even by washing for several months in changes of distilled water. Macallum perceived the necessity of substituting for pyrogallic acid, which gives colored compounds with both the phosphomolybdate and the molybdate of ammonium, some reagent which would discriminate between these two compounds, and give a color reaction with phosphomolybdate alone. This condition he found to be fulfilled by zinc chloride, previously introduced for this purpose by Polacci ('94), which gave a green color with the phosphomolybdate but did not act on ammonium molybdate. Owing, however, to the fact that zinc chloride acted very slowly, he finally adopted as a reducing agent phenylhydrazin hydrochloride, which, according to him, made a very marked distinction, in the absence of alcohol or of caustic alkali, between the molybdate and the phosphomolybdate compounds. It gave with the former in powder the brown oxide at once, in solution, a brownish precipitate, which appeared at once, or later, according

to the strength of the solution. On a solution of the molybdate containing nitric acid, *e. g.*, that used as the reagent for phosphoric acid, it had no apparent effect on the molybdenum compound, although, in a few minutes, a soluble, reddish, aromatic compound might be formed in the solution. On the other hand, with phosphomolybdates, either in the presence or in the absence of ammonium molybdate, or of nitric acid, or of both, it gave at once the dark green oxide of molybdenum.

Concerning the use of these reagents on tissues Macallum says: "On the molybdate and phosphomolybdate compounds distributed in animal and vegetable tissues, the phenylhydrazin hydrochloride acts as it does on these in the test-tube. It is not necessary to free the tissue preparations from ammonium molybdate." He recommends washing the preparations for a minute or two in a dilute solution of nitric acid after which they are transferred to the reducing solution, which in less than two minutes, brings out the green color where the phosphomolybdate compound occurs, but a faint yellow reaction where ammonium molybdate alone is present.

The technique of the reaction is as follows: Fresh tissues or tissues hardened in alcohol were used. Pieces of tissue or thin sections in the case of hardened material, were placed, for a period varying from ten minutes to forty-eight hours, in a solution of ammonium molybdate in nitric acid, prepared by dissolving one part of pure molybdic acid in four parts of strong ammonia, and adding thereto, slowly, fifteen parts of nitric acid, sp. gr. 1.2. After the nitric molybdate reagent has acted for a sufficient length of time, the preparations are washed in water or in dilute nitric acid, and treated with a ¼ per cent. solution of phenylhydrazin hydrochloride, which reduces the phosphomolybdate to a green-colored oxide of molybdenum.[1] The tissues may be then dehydrated, cleared in oil of cedar and mounted in balsam.

According to Macallum, inorganic compounds of phosphorus

[1]Although Macallum speaks of a green oxide of molybdenum being formed by this reaction, it is probable that the bodies formed belong to the blue oxides. The green color obtained at the beginning of the reduction of phosphomolybdate of ammonium in vitro is due to the yellow background of unreduced molybdate, that obtained in the tissues to the associated xanthoproteic reaction (vide infra).

are first affected, then lecithins, and finally the organic compounds of phosphorus. Where it is desired to demonstrate the distribution of the latter he recommends the preliminary removal of the lecithins by repeated extraction with hot ethyl alcohol in a Soxhlet apparatus.

In the original form devised by Lilienfeld and Monti or in the form of Macallum's modification this reaction for phosphorus has been extensively employed. Macallum's original paper dealing with the reaction contains a considerable number of contributions dealing with the distribution of organic compounds of phosphorus in various tissues, and the reaction has been applied to the solu tion of special problems of this nature by Sherrington ('94), Gourlay ('94), Scott ('99), Held ('95), Bensley ('03), Wager ('05), Richter ('05), and many others. It is therefore of the utmost importance that every detail of the reaction should be carefully tested to exclude all possible sources of error.

Recently I have obtained results which have led me to suspect that the reaction obtained by Macallum's method is not wholly due to the formation in the tissue of ammonium phosphomolybdate, but that other compounds of molybdenum may be present which are capable of reduction to the blue oxide by means of phenylhydrazin hydrochloride. For example, I observed that the peripheral portions of sections gave uniformly a deeper and more diffuse reaction than the central portions. This result was first noted in the tips of the villi in sections of the small intestine, and was ascribed to the presence of inorganic phosphates absorbed from the food. Later it was noted that the same result was obtained in sections of the liver, pancreas, and other organs. Furthermore, I observed that freshly prepared solutions of the nitric molybdate often gave a strong reaction in the tissues after very short periods of immersion. For example, sections of the fundus region of the stomach of the rabbit, treated with warm, freshly prepared nitric molybdate reagent for ten minutes, then washed in water and reduced by means of a one per cent. solution of phenylhydrazin hydrochloride gave a diffuse bluish green reaction together with a strong reaction in the nuclei and in the granules of the parietal cells. This result was clearly not due to phosphorus compounds of any sort, because the same nitric

molybdate reagent after having been kept several days, during which it had deposited copious crusts of molybdic acid, gave, when applied to sections from the same source, no such result, the reaction proceeding in the slow progressive manner characteristic of the phosphorus reaction as described by Macallum. Again, in his investigation of the nature of the granule cells of Paneth, Mr. Klein, working under my direction, found it necessary to employ formalin in the fixation of the tissues in order to preserve the granules. In preparations of this material treated by Macallum's method, we were surprised to obtain a strong reaction in the fibrils of the collagenic tissue of the tela submucosa.

Clearly, the intense marginal reaction obtained in sections and the early reaction obtained when freshly prepared solutions of the nitric molybdic reagent were used could not be due to phosphorus. These anomalous characters of the reaction as applied to sections could only be explained on the assumption that, after the treatment with the nitric molybdic reagent, there existed in the tissue compounds of molybdenum other than phosphomolybdates, which gave the blue reaction with phenylhydrazin hydrochloride.

On account of the fact that the difference between freshly prepared and older solutions of the nitric molybdic reagent is to be found in the amount of molybdic acid contained, it seemed probable that the extraordinary results of the reaction, as described above, were due to the absorption of this substance by the tissue elements. Accordingly, I undertook experiments to determine the behavior of solutions of molybdic acid in reaction with phenylhydrazin hydrochloride, as well as the capacity of the tissue elements for absorbing it from its solutions. Later it was found necessary to reinvestigate the reaction obtained by treating ammonium molybdate in solution, and phosphomolybdate of ammonium suspended in water, respectively with solutions of phenylhydrazin hydrochloride.

Although the experiments were started with the expectation that a portion of the reaction would be found to be due to absorbed molybdic acid, I still thought at that time that the fundamental assumption was true, upon which the reaction was based, namely, that the organic phosphorus was liberated from its com-

binations by the reagent, converted into orthophosphoric acid and immediately precipitated *in situ* as ammonium phosphomolybdate. As a result of the experiments, however, I have been forced to the conclusion that the whole of the reaction obtained by Macallum's method is due to compounds of molybdenum other than phosphomolybdate and that the phosphorus of the tissues is not concerned in the production of the reaction at all.

For the purpose of testing the reactions of molybdic acid with phenylhydrazin hydrochloride, I prepared two soluble molybdic acids. The first of these was prepared by the method recommended by Ullik ('67). Barium molybdate, prepared by precipitating a warm solution of ammonium molybdate with barium chloride washing thoroughly with hot distilled water, and drying the precipitate on a water bath at 100° C., was suspended in water and decomposed with its equivalent of sulphuric acid. The solution was then filtered, tested for barium, sulphuric acid and chlorides, from which it was found to be free, and the total acidity was determined by titration with a normal solution of sodium hydroxide, using phenolphthalein as indicator. Assuming that the solutions contained a molybdic acid having the formula $H_2Mo_2O_7$ the concentration of the solutions obtained by the method described above was in the case of one preparation 5.25 per cent., in another 7.52 per cent. The second molybdic acid was colloidal molybdic acid prepared by the process recommended by Graham (64), except that ammonium molybdate was employed instead of sodium molybdate. A solution of ammonium molybdate in hydrochloric acid was dialyzed for several days against distilled water, until free from chloride. The resulting solution was then titrated against normal soda solution using phenolphthalein as indicator. According to Sabanejew ('90) the molecular weight of the molybdic acid prepared by Graham's method as determined by lowering of freezing point is 620 corresponding to the formula $H_2Mo_4O_{13}$. Assuming that the same compound was obtained by the dialysis of the solution of ammonium molybdate in hydrochloric acid the solution obtained contained 6.73 per cent. of colloidal molybdic acid. From these solutions were prepared the various solutions mentioned in the succeeding experiments.

Solutions of both molybdic acids give, when treated with phenylhydrazin hydrochloride an immediate blue reaction which gradually deepens in color and a blue precipitate forms.

Sections of tissues fixed in alcohol, cut in paraffin, and fastened to the slide by the water method were placed in each of the solutions of molybdic acid. From time to time sections were removed from the solution, rinsed in water, and tested with a 1 per cent. solution of phenylhydrazin hydrochloride. It was found that the molybdic acid was taken up by the tissues from both solutions and was detectable in them by the blue reaction obtained by reduction with phenylhydrazin hydrochloride. In sections treated with pure solutions of soluble or of colloidal molybdic acid the strongest reaction was obtained in the collagenic fibrils which were deep blue. A slight diffuse reaction was obtained in the cytoplasm of cells, and a somewhat stronger reaction in the nuclear chromatin. The amount, however, of molybdic acid taken up from dilute pure solutions was not great except as regards the collagenic tissue. The experiments show, however, that molybdic acid may be taken up from its solutions by tissues and may be detected in these by the blue reaction produced by treatment of the sections with phenylhydrazin hydrochloride.

Under the conditions of the Lilienfeld-Monti-Macallum reaction molybdic acid occurs in the solution associated with nitric acid as well as with ammonium molybdate, ammonium nitrate, and the products of dissociation of all these compounds. Accordingly, the effect of the presence of acids on the absorption of the molybdic acid from its solutions by tissues was tested. Sections were placed in solutions of soluble and of colloidal molybdic acid to which five per cent. of nitric or of hydrochloric acid had been added, and were tested from time to time with phenylhydrazin hydrochloride. I found that the addition of either nitric acid or hydrochloric acid to the solutions of molybdic acid produced a remarkable increase in the capacity of sections for combining molybdic acid, which was again detectable by the blue or green reaction obtained by reduction with phenylhydrazin hydrochloride. With the mixture of nitric and molybdic acids the reaction obtained after reduction was a deep greenish blue color in the nuclear chromatin, a faint greenish blue in the cytoplasm,

and a deep blue in the collagenic fibers. Except for the strong reaction in the connective tissue, the result obtained by treatment of sections with a solution of molybdic acid containing nitric acid, followed by reduction in one per cent. phenylhydrazin hydrochloride was exactly similar to the so-called phosphorus reaction obtained by the procedure recommended by Macallum. It may be noted that the color obtained by the use of molybdic acid containing nitric acid followed by reduction differed from that produced by reduction of molybdic acid by phenylhydrazin in the test tube, inasmuch as the former gives a greenish blue color, the latter a pure blue. This difference was obviously due to the yellow background afforded by the xanthoproteic reaction. As in the phosphorus reaction, the absorption of the molybdic acid was progressive, the reaction after eighteen hours being much stronger than after three hours.

Similar results were obtained with solutions of molybdic acid containing hydrochloric acid, except that the molybdic acid was taken up much more rapidly from the hydrochloric solution than from the nitric solution, and that the resulting reaction was blue rather than greenish blue, owing to the absence of the yellow zanthoproteic reaction. A further difference exhibited itself in the fact that sections left for some time in the solutions developed the blue color *without the use of any reducing agent*, the organic compounds of the tissue evidently acting as reducers. In the presence of nitric acid this, of course, could not occur because of the strong oxidative action of this compound.

Thus, in sections treated with solutions of molybdic acid containing either hydrochloric, or nitric acid, followed by phenylhydrazin hydrochloride, results were obtained which were the exact counterpart of the results of the so-called phosphorus reaction, although there could be little possibility of the formation of precipitates of ammonium phosphomolybdate in the tissues. Curiously enough, the anomalous characters occasionally observed in the reaction obtained by Macallum's method were to be found in the molybdic acid material, that is to say, the more intense diffuse reaction of the outer portions of the sections and the deep reaction in the connective tissue. It seemed clear from these experiments that a portion, at least, of the result

obtained by the procedure of Macallum was due to absorption of molybdic acid from the nitric molybdate solution. There was, however, some possibility that even this reaction with molybdic acid solutions depended on the presence of phosphorus and its liberation as phosphoric acid. It might be supposed that this phosphoric acid reacted with the molybdic acid to produce phosphomolybdic acid which was in turn precipitated by the albumens of the tissues. Or it might even be supposed that ammonium phosphomolybdate was formed, the ammonium ions necessary to the reaction being furnished by the albumens. That this is not the case, and that the reaction obtained by the use of molybdic acid solutions is in no way dependent on the phosphorus content of the tissue, I think the following experiments will show.

In studying the reaction of solutions of molybdic acid with phenylhydrazin hydrochloride, I found that the addition of nitric acid to the mixture retarded the reaction and if a sufficient quantity were present prevented it altogether. Accordingly, experiments were undertaken to determine the limits of this reaction with molybdic acid, ammonium molybdate, and ammonium phosphomolybdate, respectively, in the hope that a sufficient difference in the behavior of these compounds would be discovered to enable one to employ a solution of phenylhydrazin hydrochloride containing enough nitric acid to inhibit the reduction of molybdic acid and of ammonium molybdate while permitting the reduction of ammonium phosphomolybdate, and thus discriminate between these compounds occurring in tissues treated by Macallum's methods.

I found that with a constant concentration of phenylhydrazin hydrochloride, the amount of nitric acid required to prevent the reduction to the blue oxide of molybdenum varied directly with the concentration of the molybdic acid but was constant for any given concentration.

I found, moreover, that the blue reduction was invariably obtained in solutions of ammonium molybdate containing nitric acid, when they were treated with solutions of phenylhydrazin hydrochloride, provided the amount of nitric acid did not exceed a certain amount, which, as in the case of molybdic acid, was

constant for a given concentration of the other two constituents but varied directly with the concentration. This fact made it necessary to determine the conditions of this reaction with ammonium molybdate if solutions of the hydrochloride containing nitric acid were to be used for the reduction of the sections, because in trying to eliminate the reduction of the molybdic acid by using a solution of the hydrochloride containing nitric acid, a new source of error might be introduced, inasmuch as the nitric acid would make ammonium molybdate available to the blue reduction.

The experiments to determine the limits of these reactions were made in test-tubes in much the same way as experiments in hæmolysis are carried out. In each of a series of test-tubes was placed, from a pipette graduated in fiftieths of a cubic centimeter, a measured quantity of the solution of molybdic acid. To this was added a quantity of nitric acid solution of known strength increasing by increments of one tenth of a cubic centimeter from tube to tube. Sufficient distilled water was then added to make the contents of each tube up to 9.5 c.c., and finally 0.5 c.c. of a two per cent. solution of phenylhydrazin hydrochloride was added to each tube. In such a series of tubes, if a sufficient range of concentrations of the nitric acid was included the tubes at one end of the series would give the blue reduction, those at the other would at first show no signs of reaction, but after several hours would develop a brownish color. At some point in the series two tubes, side by side, differing from one another only in the nitric acid content, would present the one a blue, the other a brown color. When solutions of nitric acid containing 32 grammes of nitric acid per 100 c.c. were employed, that is, when the difference in the contents of two tubes amounted to .032 gr. of nitric acid, the contrast in color between the tubes which marked the limit of the reaction was a very striking one. Attempts to define this limit more accurately by the use of more dilute solutions of nitric acid, and accordingly smaller increments, of nitric acid from tube to tube did not result more satisfactorily. For example in a series of tubes in which the increment of nitric acid was 0.012 gr. from tube to tube, the transition was distributed over several tubes, those immediately preceding the first

tube free from the blue precipitate containing a slight blue precipitate which subsided after several hours leaving a brownish supernatant fluid. Thus, the possible error in determining the proportion of nitric acid necessary to prevent the blue reaction is considerable, although the results are accurate enough for the purposes of this investigation, as subsequent statements will show.

Reactions of Soluble Molybdic Acid with Phenylhydrazin Hydrochloride in the Presence of Nitric Acid.

Strength of Molybdic Acid in Fractions of Normal.	Percentage of Phenylhydrazin Hydrochloride.	Percentage of Nitric Acid Necessary to Prevent Blue Reaction.
0.02	0.1	1.38
0.04	0.1	2.26
0.06	0.1	2.94
0.08	0.1	3.27

Reactions of Solutions of Ammonium Molybdate with Phenylhydrazin Hydrochloride in the Presence of Nitric Acid.

Solutions of ammonium molybdate when treated with phenylhydrazin hydrochloride give, as stated by Macallum, a brown color, and a brown precipitate slowly forms in the solution. In the presence of nitric acid, however, provided the latter does not exceed a certain amount which varies with the concentration of the molybdate, the reaction consists in the production first of a blue color and finally of a deep blue precipitate. If the amount of nitric acid exceeds this maximum no reaction occurs at first, but a brown color slowly develops in the solution. The following table gives the concentration of nitric acid necessary to prevent wholly the blue reaction with several concentrations of molybdate.

Percentage of Ammonium Molybdate.	Percentage of Phenylhydrazin Hydrochloride.	Per Cent. Nitric Necessary to Prevent Blue Reaction.
0.5	0.1	3.14
1.0	0.1	4.40
1.5	0.1	5.03
2.0	0.1	5.66

Reactions of Phosphomolybdic Acid and Phosphomolybdate of Ammonium with Phenylhydrazin Hydrochloride.

Phenylhydrazin when added to a solution of phosphomolybdic acid or to crystals of ammonium phosphomolybdate suspended in water gives at once the reduction to the blue oxide. The reaction under these conditions proceeds so rapidly that it is difficult to follow the steps. The resulting body is not green in color as described by Macallum but blue, the green color which is first seen when phenylhydrazin hydrochloride is added to the crystals of ammonium phosphomolybdate being simply due to the yellow background afforded by the unreduced phosphomolybdate. After a few minutes the reaction proceeds still further and a soluble blue-violet compound is formed.

In the presence of nitric acid, the reaction proceeds somewhat more slowly, although it is less sensitive to the presence of nitric acid than the corresponding reactions with molybdic acid and ammonium molybdate. With crystals of phosphomolybdate suspended in water and 0.1 per cent. of phenylhydrazin the concentration of nitric acid may be increased to 36 per cent. without the reduction to the blue oxide being prevented. Even in the presence of this great amount of nitric acid the reduction of the phosphomolybdate reaches a maximum within ten minutes after the phenylhydrazin hydrochloride is added. The reaction between phenylhydrazin and phosphomolybdic acid proceeds in much the same way, and is similarly much less sensitive to the presence of nitric acid, than the corresponding reactions with molybdic acid and ammonium molybdate. For purposes of comparison the experiments were made the results of which are presented in the subjoined table.

Percentage of Phosphomolybdic Acid.	Percentage of Phenylhydrazin Hydrochloride.	Per Cent. Nitric Acid Necessary to Prevent Blue Reaction.
0.1	0.1	6.55
0.2	0.1	9.82
0.3	0.1	13.10
0.4	0.1	14.74

On comparison of this table with the preceding one it will be seen that the reduction of phosphomolybdic acid having a con-

centration of 0.1 per cent. will proceed in the presence of nitric acid having a concentration sufficient to prevent reduction in a solution of ammonium molybdate of 2 per cent. strength.

While it would have been difficult to draw from these experiments conclusions as to the probable behavior of the compounds of molybdic acid in the tissues when treated with solutions of phenylhydrazin hydrochloride containing nitric acid, yet they suggested a possibility which was capable of being proved experimentally that the compounds of molybdic acid and molybdates found in the tissues would fail to react to phenylhydrazin hydrochloride in the presence of an amount of nitric acid which would have no effect on the reduction of ammonium phosphomolybdate.

In order to test this question, sections of the liver of Necturus prepared after fixation in alcohol and fastened to the slide by the water method were treated with Macallum's nitric molybdate reagent, a solution of soluble molybdic acid in 10 per cent. nitric acid, and a ten per cent. solution of phosphoric acid, respectively. The two first mentioned solutions were allowed to act for three hours at 37.5° C. followed by eighteen hours at ordinary room temperature. They were then tested with a 0.1 per cent. solution of phenylhydrazin hydrochloride and found in each case to give a strong reaction corresponding in its characters and distribution to the phosphorus reaction of Macallum. The reaction obtained in the sections treated with the solution of molybdic acid was much the stronger. Other sections from the same lot were then treated with solutions containing 0.1 per cent. of phenylhydrazin hydrochloride and varying known quantities of nitric acid, in each case for a period of fifteen minutes. The sections from the molybdic acid solution and from the nitric molybdate reagent were treated side by side in the same solution, and for purposes of control a section which had been soaked in phosphoric acid and then treated with the nitric molybdate reagent was also put at the same time in the solution, so that it was possible to observe the effect of different concentrations of nitric acid on the reduction of sections treated with molybdic acid, or with the nitric molybdate reagent, and of sections containing ammonium phosphomolybdate artificially introduced. It was my expectation that a low concentration of nitric acid would suffice to abolish that portion of the reac-

tion which was due to ammonium molybdate and to molybdic acid, and that a considerable residuum of the reaction would be found unaffected by even high concentrations of nitric acid and could thus be interpreted as a true phosphorus reaction. I was quite unprepared for, and greatly disappointed at the actual result of these experiments, namely, that relatively low concentrations of nitric acid abolished the reaction altogether.

With a concentration of 3.27 per cent. of nitric acid, phenylhydrazin hydrochloride 0.1 per cent., the molybdic acid sections and the nitric molybdate section showed no reaction after three minutes' treatment, although the section containing ammonium phosphomolybdate artificially introduced gave a maximum reaction in less than one minute. After fifteen minutes' action, a very faint reaction was obtained in the nuclei, both in the molybdic acid section and in the nitric molybdate section. When the concentration of the nitric acid reached 16.37 per cent., the phenylhydrazin remaining the same, the reaction was not recognizable after fifteen minutes' treatment although sections containing ammonium phosphomolybdate artificially introduced reduced to a maximum depth of color in the same solution in five minutes. I have repeated these experiments many times, always with the same results. It is significant that the reaction disappeared at exactly the same point as regards concentration of nitric acid in the molybdic acid section and the nitric molybdate section.

Only one conclusion is possible from these experiments, namely, that sections after treatment with Macallum's reagent for this length of time did not contain appreciable quantities of ammonium phosphomolybdate. Thus the fundamental assumption on which the reaction of Lilienfeld and Monti and of Macallum is based falls to the ground. It is obvious that if the phosphorus of the organic compounds is liberated at a point short of the destruction of the recognizable structures of the cell, it is not, at all events, precipitated *in situ* by the nitric molybdate reagent.

As a result of these experiments I am of the opinion that the reaction obtained by Macallum's procedure is entirely due to the formation of compounds of molybdic acid with the albumens of the tissue and not in any respect to the formation of ammonium phosphomolybdate at the expense of the organic phosphorus.

The facts on which the conclusion is based are, briefly, as follows :

The essential conditions of a successful phosphorus reaction are, first, that the phosphorus may be liberated from its organic combinations at a point short of the destruction of the recognizable structure of the cell ; second, that the liberated phosphorus be precipitated at once at the point of origin as ammonium phosphomolybdate ; third, that the reducing substance employed to make the phosphomolybdate visible for microscopic study act on phosphomolybdate and on no other compound of molybdenum which may be present in the tissue.

Phenylhydrazin hydrochloride does not meet the third condition because it reduces to the blue oxide of molybdenum, soluble molybdic acid in the test tube as well as molybdic acid combined with the tissue constituents in sections.

Phenylhydrazin hydrochloride also produces the blue oxide when treated with ammonium molybdate in the presence of nitric acid, provided that the latter does not exceed a certain concentration which is constant for constant concentrations of the molybdate.

Nitric acid affects the reduction of molybdic acid, ammonium molybdate, and ammonium phosphomolybdate, by phenylhydrazin hydrochloride in the same way, namely, retards the reduction, but to different degrees, inasmuch as low concentrations of nitric acid *prevent* the reduction of the two former to the blue oxide, while high concentrations of nitric *merely retard* the blue reduction of the phosphomolybdate. Accordingly, if phosphomolybdate is formed at the site where a reaction is obtained by the method of Macallum, the reaction ought to be elicited by treatment of the sections with solutions of phenylhydrazin in having a high content of nitric acid. This, however, the experiments show is not the case. Even low concentrations of nitric acid eliminate the greater portion of the reaction, and the reaction is entirely abolished by a nitric acid content which has little effect on the reduction of phosphomolybdate of ammonium artificially introduced into sections for purposes of control. Furthermore, the reaction is abolished at the same concentration of nitric acid with sections treated with Macallum's nitric molybdate reagent

as with sections treated with a pure solution of molybdic acid. These facts dispose finally of the first and second essential conditions of a successful microchemical reaction for organic phosphorus, for it is clear that if the sections after treatment with the reagent contain no phosphomolybdate of ammonium, that the organic phosphorus has either not been liberated from its compounds, or that, if it has, it has not been precipitated at the moment and at the point of liberation. If these conclusions are correct, it is also obvious that there is no hope of a real phosphorus microchemical reaction being obtained by the employment of the nitric molybdate reagent.

These conclusions do not, of course, apply to the identification of phosphates by the nitric molybdate reagent, in cases where the characteristic crystal form of the ammonium phosphomolybdate can be recognized under the microscope.

In conclusion, it may be mentioned that in making the experiments to determine the effect of nitric acid on the reduction of the molybdenum compounds by phenylhydrazin hydrochloride it is important to employ solutions which are free from nitrous acid, which reacts with the phenylhydrazin and reduces its concentration.

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ON THE LOCOMOTION OF A SEA ANEMONE (METRIDIUM MARGINATUM).[1]

J. F. McCLENDON.

Last winter while studying some animals in the marine aquaria of the University of Pennsylvania I noticed that the anemones, after being placed at the bottom of an aquarium, would creep up the side of the glass to a more favorable position. Their method of progression is similar to the ordinary creeping of a snail,[2] con-

FIG. 1. *Metridium marginatum* seen through the glass, up which it is creeping. The lower side of the photograph has been outlined.

sisting of a succession of waves that travel from behind forward, but in the anemone the waves are larger and not so rapid or regular. The accompanying photographs were taken of an anemone creeping up the side of an aquarium, with its distal end inclined forward, probably to test the water into which it was advancing. The undulations of the foot progress in the direction of locomotion. If the functionally posterior end of the

[1] Contribution from the Zoölogical Laboratory of the University of Pennsylvania.

[2] For a more complicated form of locomotion in some snails see A. J. Carlson: "The Physiology of Locomotion in Gasteropods," BIOL. BULL., Vol. 2, January 1905, pp. 85–93.

foot be watched closely it will be seen to let go at several points (below in Fig. 1) and slip forward. This contraction is carried forward and on reaching the center of the foot, the contracted portion rises up from the glass, forming a wave that deepens as

FIG. 2. *Metridium marginatum* about half a minute later than Fig. 1.

it approaches the "anterior" end (Fig. 2, above). On reaching the "anterior" edge the wave is retarded by the firmer attachment of the edge, which releases locally, breaking the wave into segments (Fig. 1, above). A wave requires about a minute to traverse the foot of the anemone, and before it has disappeared, another commences.

I threw a number of anemones into an aquarium to observe their actions. They threw out acontia, which caught hold of any solid near them and contracted until some portion of the foot touched the object and caught hold. One anemone sinking to the bottom and resting on its tentacles contrived to right itself by suddenly contracting and expelling water from its mouth. I observed this once or twice subsequently, but rather think it a coincidence than a common reflex.

BIOLOGICAL HALL, UNIV. OF PENN.,
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THE SIGNIFICANCE OF SCUTE AND PLATE "ABNORMALITIES"[1] IN CHELONIA.

A Contribution to the Evolutionary History of the Chelonian Carapace and Plastron.

H. H. NEWMAN.

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I. Introduction.

1. *Statement of the Problem.*

During a residence of several years on Lake Maxinkuckee, Marshall County, Indiana, my attention was repeatedly attracted to the large numbers and variety of species of tortoises that are found in the lake and in its accessory streams, swamps and pools.

In the spring of 1903 I began a study of the habits, variations,

[1] The word "abnormalities" in the title is used for lack of a better one, and includes supernumerary scutes and plates, deficiencies in these structures and cases of fusion. The word "diversities" might have been used with equal appropriateness.

etc., of these species, involving the collection of large numbers of individuals of all sizes. One of the most striking phenomena that came to light was the prevalence of many kinds of scute abnormalities, consisting for the most part of supernumary scutes on the carapace and plastron. As examples multiplied I became aware of a marked degree of regularity in these abnormalities, the same supernumerary scutes occurring in exactly the same locations time after time.

Diversity in scutes had been noted by two observers. Gadow ('99) studied *Thalassochelys caretta* (L.), a species with no fixed number or arrangement of scutes, and Parker ('01) found two abnormal specimens of *Chelopus insculptus* (Le C.) on the basis of which he published a paper on correlated abnormalities in the scutes and the bony plates.

It seemed, then, that this phenomenon needed further investigation and the collection of large numbers of abnormal specimens was begun in the hope of reaching a rational explanation of this very prevalent diversity. Careful study has convinced me that these abnormalities are to be considered not as meaningless anomalies but as examples of systematic atavism in the sense of deVries. From this standpoint it seems possible to throw some light on the phylogeny of *Chelonia*.

The color patterns are intimately associated with the scutes and throw much light on their phylogeny. Consequently a brief consideration of chelonian coloration has been appended.

2. *Nomenclature. The Normal Plate and Scute Pattern.*

The following description and appended drawings (Plate I., Figs. 1 and 2), although referring particularly to an adult female specimen of *Graptemys geographica*, will apply to any genus of the Emydidæ. Fig. 1 represents the dorsal and Fig. 2 the ventral aspect.

The armor of tortoises consists of two elements, bony plates and horny scutes, which for brevity will be referred to as *plates* and *scutes*. Dotted outlines are used for the plates and solid outlines for the scutes. In labeling, small letters are used for plates and capital letters for scutes.

A. Plates. — There are in the *carapace* (Fig. 1) five longitudinal

rows of plates, a single median and two paired rows. The median row has been variously designated as dorsal, vertebral and neural. In this paper the term *neural* will be invariably used. The neural row consists of the following elements : an anterior plate of large size called the nuchal (*nu.*), eight neurals (*n.* 1–8), two procaudals (*pr.* 1 and 2), and posteriorly the pygal (*p.*).

Lateral to the median row are the paired costals (*c.* 1–8), directly overlying the eight pairs of ribs.

Bordering the carapace on both sides and extending from nuchal to pygal are the marginals (*m.* 1–11).

The plates of the *plastron* (Fig. 2) are nine in number — the paired epi- (*e.*), hyo- (*ho.*), hypo- (*hp.*), and xiphi- (*x.*) plastrals, and the unpaired endo-plastral (*en.*).

The hyo- and hypoplastrals articulate directly with the fourth, fifth and sixth marginals and form the so-called " bridge " between the dorsal and ventral armor.

B. Scutes. — On the *carapace* (Fig. 1) there are, as in the case of the plates, five longitudinal rows of scutes that receive the same names as the plates. The median row, *neurals*, consists of a small anterior element, the nuchal (*NU.*), and five large *neurals* (*N.* 1–5). There are four pairs of large *costals* (*C.* 1–4). Twelve pairs of *marginals* (*M.* 1–12) completely surround the carapace with the exception of the small space occupied by the nuchal.

The scutes of the plastron (Fig. 2) are twelve in number, consisting of six pairs of large flat elements named from anterior to posterior end as follows : gulars (*G.*), humerals (*H.*), pectorals (*P.*), abdominals (*A.*), femorals (*F*), and anals (*AN*).

At the angles made by the junction of the pectorals and abdominals with the marginals are two pairs of small triangular scutes called respectively axillaries (*X.*) and inguinals (*I.*). These constitute all that remains in the Emydidæ of the *inframarginals*, a row much more prominent and complete in more primitive families.

No other plates or scutes occur normally among the Emydidæ, but for the sake of completing the nomenclature, it should be mentioned that one species, *Macrochelys temmincki*, possesses an additional pair of rows of scutes between costals and marginals,

called *supramarginals*. Traces of a median ventral row of scutes are found normally in some species — and I have given the name "*interplastral*" to this row. A single median scute occurs normally in the anterior part of the plastron of certain specialized groups and receives the name *intergular*.

II. Discussions of the Present Status of the Question Concerning the Morphology of the Chelonian Armor.

The frequent abnormal occurrence of traces of the inframarginals and interplastrals in *Graptemys geographica*, *Chrysemys marginata* and *Chelydra serpentina* led me to review the literature relating to the evolutionary history of the chelonian carapace and plastron.

For nearly a century the chelonian armor has offered to morphologists a problem of unusual difficulty, and, although much has been written on the subject, its derivation is still unsettled. The question has been attacked from the three standpoints of paleontology, embryology and comparative anatomy.

1. *Paleontological* data are far from conclusive. It is not possible to go into this phase of the subject at all fully. Baur in 1887 published a brief summary of the more valuable paleontological data in an article entitled "On the Morphogeny of the Carapace of the Testudinata." A brief statement of the substance of this paper will, perhaps, serve to show the inadequacy of the paleontological evidence in this case.

The condition seen in the Dermochelydæ is considered to be the most primitive. Fossil remains of this group agree closely with the existing *Dermochelys coriacea* in the possession of "a pavement of small osseous plates extending over the whole shield, jointed to one another by more or less fine sutures. The number of these plates is much larger than that of the other Testudinata, which is never more than 70." This pavement of osseous plates is not united with the internal skeleton, as are the plates of other Testudinata, but has an independent dermal origin. "That the carapace of the Dermochelydæ is homologous to the carapace, without internal skeleton, of the rest of the Testudinata, there is no doubt." The fusion of the dermal pavement bones with the ribs and vertebræ is, according to Baur, proved by a

specimen of *Eretmochelys imbricata*, a fossil species in which are found "small polygonal plates of the same shape as those of Dermochelys, suturally connected with the third, fourth, fifth and sixth costal plates." "A form between the Dermochelydæ and "*Thecophora*" (Dollo) is represented by the oldest known turtle, *Psephoderma alpinum*, H. v. Meyer, from the Triassic of the Bavarian mountains, preserved in Munich. In this highly interesting specimen, never mentioned in monographs on the Testudinata, we have certainly not less than 193 plates suturally united." According to Zittel's Paleontologie, Baur later expressed the opinion that *Psephoderma* may not be a chelonian at all, but perhaps a nothosaurus. Thus doubt is cast upon the best link in the chain of evidence. That all the principal groups of *Chelonia* were in existence in the earlier Mesozoic ages and that Palæozoic *Chelonia* are entirely unknown are familiar facts. So our attempts to reconstruct an ancestral condition must be made largely on the basis of embryology and comparative anatomy.

2. Nor is *embryological* evidence of chelonian phylogeny at all conclusive. The best and most recent study of the developmental history of the chelonian carapace and plastron was made by Goette in 1899. He summarizes the previous literature on the subject and shows that the main question at issue is that of the character of the neural and costal plates. Some authors, principally paleontologists, have maintained that these structures have a dermal origin and hence arise independently of the internal skeleton. Others hold that these plates are mere outgrowths of the ribs and spinal processes of the vertebræ. Goette favors the latter view and presents as evidence of its correctness a series of very careful embryological studies.

Suspecting that there might be some flaw in Goette's work, I repeated much of it, using the embryos of *Chelydra serpentina* and *Graptemys geographica*, and have satisfied myself that the neural and costal plates actually do originate as outgrowths of a differentiated tissue that surrounds the neural and rib cartilages. Whether this differentiated tissue be true periosteum, as Goette affirms, or simply a somewhat denser portion of the connective tissue that fills the space between the epidermis and the cartilagi-

nous skeleton, is not certain. Haycraft ('99) maintains the latter view, but his paper is far from convincing.

As to the remaining plates of the carapace — nuchal, procaudals, pygal and marginals — there is no difference of opinion. All agree that they are of true dermal origin.

Thus it would seem that the plates of the carapace have a dual origin — the neurals and costals being periosteal ossifications while the nuchal, procaudals, pygal and marginals are dermal ossifications.

The carapace, then, as it exists to-day is not a simple structure but consists of a complex of at least two independent systems of bones.

Accepting the evidence of embryology as to the origin of the neural and costal plates, it remains to determine whether the dermal ossifications are, as Goette believes, mere supplementary structures that have come in to supply the deficiencies of the periosteal system, or are remnants of a once more or less complete dermal carapace that has in large measure been rendered superfluous by the broadening-out of the ribs and neural processes. The latter view would involve the former existence of complete rows of dermal bones overlying the vertebræ and ribs. Embryological evidence seems contrary to this view, as no dermal ossifications are found in the costal or mid-neural regions. It is possible that we may in this case overestimate the evidence of embryology as a guide to phylogeny. The great antiquity of the chelonian carapace is undoubted and in highly specialized structures that have attained a marked morphological fixity we should not be surprised to find great condensation in development, so that two structures formerly independent in origin — such as dermal and periosteal plates — may originate simultaneously so as to form only one inseparable structure. It seems quite plausible, then, that the rapid secondary broadening of ribs and neural processes has crowded out or appropriated the primordia that formerly went to form the dermal carapace and that only in places where the ribs and neural processes fail to reach the dermis do the true dermal bones have a chance to appear.

The fact that the nuchal plate appears before the ribs and

neural spines have even commenced to broaden out and that the procaudals and the marginals follow before the neurals and costals are completely organized, points to the antiquity of these dermal structures and indicates that the neurals and costals are of more recent origin.

3. *Comparative anatomy* furnishes us much valuable evidence. In the family Trionychidae, for example, we have a series of forms that show a gradual reduction of a portion of the dermal armor. Fossil Trionychidæ are well known in which are shown a nuchal, a procaudal and a nearly complete set of marginal plates. Such a form was figured by Dollo in 1884 and named by him *Pseudotrionyx*. Another fossil species discovered by the same palæontologist and named by him *Emyda granosa*, lacks the procaudal and the marginals from the anterior half of the carapace. A third form, *Emyda Ceylonensis*, possesses a nuchal and several marginals at the posterior part of the carapace. The extreme limit of reduction is seen in *Aspidonectes spinifer*, which possesses only the nuchal plate as the last remnant of the dermal carapace.

It will be noted that the order of the appearance of these dermal ossifications in ontogeny is just the inverse of the order of disappearance in phylogeny. The latest elements to be formed in ontogeny are the first to disappear in phylogeny. This is just what we would expect if we consider that there has been a gradual shortening of the developmental process, a gradual elimination of the latest stages. The Trionychidæ show clearly that there is a marked tendency to reduce the system of dermal bones and it is not difficult to imagine that earlier reduction has taken place in which the dermal ossifications of midneural and costal regions were lost.

What evidence have we that such dermal ossifications overlying neural processes and ribs actually existed? O. P. Hay ('97) in an important paper dealing with the evolution of the chelonian carapace and plastron, describes and pictures an incomplete carapace of a fossil form named *Toxochelys serrifer*. Three ossicles occur above and overlapping the neural plates and occupy positions coincident with the keels of the second, third and fourth neural scutes. These ossicles have the general form

of the tubercles seen on the dorsal ridge of the tail of *Chelydra serpentina*, and this suggests that the ossicles of *Toxochelys* are merely a continuation forward of a series of tubercles that must have been present on the tail.

Hay suggests that the keels seen especially in the young of modern *Chelonia* are the representatives of ancient dermal tubercles that formed the chief armor of ancestral forms. That in most cases these dermal ossicles have ceased to form independently of the deeper and more vigorous bony layers is perhaps to be expected as the result of condensation in developmental processes.

The degree to which modern species exhibit keels is extremely varied. Some highly specialized forms show none, or at most one, even in very young specimens, while one very primitive species, *Macrochelys temmincki*, possesses seven distinct keels on the carapace and four rows of flat scutes on the plastron. This multiplicity of keels is evidently a very primitive condition and naturally suggests to Hay the condition seen in *Dermochelys coriacea* in which twele well-marked keels are found, each keel consisting of rows of dermal ossifications that are lager and more prominent than the remaining intermediate ossicles that form the continuous pavement of the test. This peculiar aberrant chelonian is taken by Hay, following Baur and others, as the hypothetical ancestral type from which our modern chelonians have been derived by a process of simplification.

A survey of the field reveals the fact that the nearest approach to this condition of twelve rows of keels is seen in *Macrochelys temmincki*, which possesses seven distinct keels on the carapace. The four rows of flat scutes on the plastron may once have been keeled, for keels on the plastron are known in both extinct and living groups. The total number of keels or keel equivalents in *Macrochelys* is then eleven, one short of the supposed ancestral condition. The missing keel is the mid-ventral one and is represented in certain groups by intergulars. Thus all of the ancestral keels find representatives among modern species.

Hay seems to have been the first observer to suggest the importance of the scutes as factors in the evolution of the carapace. Previous authors have confined their attention to the bony struc-

tures, considering the scutes as of little significance. Hay's view of the rôle of the scutes may be stated briefly as follows: The probable ancestral condition is that seen in *Dermachelys*, the skin of which is found to be broken up into small polygonal areas, larger in the keels than elsewhere. These areas coincided with the osteodermal plates that are or will be developed in the skin. As the deeper elements of the carapace (neural and costal plates) increased in protective efficiency, the dermal structures were in many regions rendered superfluous and disappeared. In some cases the scutes were lost with their corresponding plates, in others the lost plate left its trace in the keel of the scute. The direction of growth of each of the existing series of scutes shows the direction of encroachment on other rows now lost.

This exposition of Hay's seems to me to be the most rational yet advanced, yet I believe that he fails to appreciate the evidence of embryology and thus introduces undue complexity. In the first place, he considers the nuchal plate as a fascia bone instead of an ordinary dermal plate. In the second place he states that the neural and costal plates are of the same character as the nuchal. Embryology shows that the nuchal plate is as true a dermal bone as are the marginals, while the neurals and costals are true periosteal expansions. It seems to me more rational to suppose that the dermal ossifications of the mid-neural and costal regions have undergone a complete suppression identical with that indicated by the series of Trionychidæ described above, rather than that they have become indistinguishable by fusion with the rib and neural fascia bones, as Hay calls them.

If we remove the scutes and underlying dermis from the carapace of a specimen of *Chelydra* we find that the long tubercles on the neural and costal plates bear no constant relation to the plates themselves, but are nevertheless clearly of a piece with them. It was natural for Hay to suppose that these bony tubercles were produced separately and then fused with the underlying plates. I have been able to trace this matter to a conclusion in the young of *Chelydra*, with the result that I have seen all the stages of ossification in the carapace and know that the tubercular keels on the neural and costal plates are produced by gradual thickenings of the growing plates. These thickenings

send out branching processes that gradually displace the dermal connective tissue of the tubercles and fill the space with bone. Complete ossification of these tubercles does not occur until the animals are several years of age.

III. Description and Discussion of Abnormalities.

That a process of reduction both in the number of rows of scutes and in the number of scutes in surviving rows has taken place seems highly probable. From this standpoint I made a systematic study of all the abnormal specimens that showed traces of these lost rows or lost scutes. Inframarginals of all grades of prominence were found in specimens of *Graptemys geographica* and *Chrysemys marginata*, while interplastrals were found more rarely in the same two species. It will be noted that both of these recurring rows are plastron rows which probably means that the carapace has reached a high degree of fixity with reference to number of rows. Yet many abnormalities are found that indicate that the reduction in the number of scutes in a row was of comparatively recent occurrence.

These abnormalities will be discussed under three heads: (1) Inframarginals, (2) interplastrals, (3) supernumerary scutes in a row.

1. *Inframarginals.*

The occurrence or non-occurrence of inframarginals has formed the basis for separating the Thecophora into two great groups. Gadow in his volume on Amphibia and Reptiles gives Boulanger's key for classifying *Chelonia*. In this the two groups are characterized as follows:

1. Pectoral shields separated from the marginals by inframarginals — Chelydridæ, Platysternidæ, Cinosternidæ.

2. Pectoral shields in contact with the marginals — Testudinidæ, Chelydidæ, Pelomedusidæ.

It is evident that the more primitive families possess as normal factors this row of scutes while the more specialized families *normally* lack this row. When, however, dozens of specimens of *Graptemys* and *Chrysemys* possess this row in more or less perfect form, I am forced to consider this phenomenon as a well-marked case of systematic atavism. In view of the fact that no

such anomalies have been previously described, it seems worth while to tabulate those in my collection — an easy task in view of the fact that the scutes occur in definite places. In any species, such as *Chelydra*, that possesses this row normally, there are typically three scutes in the row, one in contact with the axillary, one at the angle of contact of the pectoral and humeral and the marginals, and one abutting on the inguinal scute. These three scutes may be designated respectively as I., II. and III. Out of 476 specimens of *Graptemys geographica* examined, I found 31 with traces of inframarginals varying all the way from three large scutes on each side to one small one on one side. The tabulation below gives the number of the specimen, the sex, the length and breadth of carapace in millimeters, the occurrences of inframarginals on the right and left sides separately. Three general sizes are distinguished, which although quite arbitrarily laid down may serve to give a more definite idea of the amount of variation that occurs. These sizes are designated as large, medium and small.

At Woods Hole this summer I found two specimens of *Nannemys guttata* and three specimens of *Chrysemys picta* with well marked inframarginals.

It will be readily seen that in both species the middle scute is much the commonest recurrence, and this is natural if we consider that in *Chelydra*, and other species with well developed inframarginal rows, the middle scute is always the largest. The largest and most vigorous scute would probably persist longer and hence be most likely to recur as an atavistic reminiscence. The fact that no. III. is next in prevalence in *Graptemys* and no. I. in *Chrysemys*, indicates that the order of suppression of the other scutes of the row was subject to individual and group variation.

In the species *Chelydra serpentina* the inframarginal row is in a highly variable condition. Many stages in the reduction of numbers of scutes are to be seen in different individuals. The middle scute, corresponding to no. II., is always the largest, and the adjoining ones are next in size and would correspond to no. I. and no. III. Frequently there are two or three smaller scutes both in front of and behind the large central scutes, but

GRAPTEMYS GEOGRAPHICA.

No.	Sex.	Length in mm.	Breadth in mm.	Right Side.	Left Side.
1	F	98	81	Small II.	Medium II.
2	F	189	139	Small II.	
3	F	200	171	Small II.	
				Large III.	
4	M	109	82		Medium I.
5	F	75	63	Large II.	Medium II.
6	?	63	57	Medium III.	Medium III.
7	M	82	70		Small II.
8	?	63	56	Large II.	
9	F	75	63	Small III.	
10	?	60	54	Small II.	Medium II.
11	?	57	51	Large II.	Large II.
				Large III.	Large III.
12	F	195	147	Large III.	Large II.
					Large III.
13	F	160	118	Medium III.	Large III.
14	F	99	82	Medium I.	Medium I.
				Medium II.	Medium II.
				Medium III.	Medium III.
15	F	94	82	Large II.	Large II.
				Large III.	
16	F	134	112	Large I.	Large I.
				Large II.	Large II.
17	F	101	76	Medium I.	Large II. (See Fig. 44.)
				Large II.	Large III.
				Large III.	
18	M	88	69	Medium I.	
19	M	95	73	Large II.	Large II.
				Large III.	
20	?	56	51	Small II.	Small II.
					Small III.
21	?	55	50	Medium II.	Medium II.
22	?	57	53	Large II.	Large II.
23	?	62	54	Large II.	Large II.
					Large III.
24	F	80	65		Medium II.
25	M	60	50	Large II.	Medium I.
					Medium II.
26	?	51	48	Medium II.	Medium II.
					Medium III.
27	?	58	53	Small III.	
28	M	66	54	Medium II.	Medium I.
29	M	110	78	Medium III.	
30	M	97	77		Small II.

they shown signs of suppression and in the majority of specimens are of insignificant size. The axillary and inguinal scutes of the Emydidæ, etc., correspond, I believe, to two of these smaller scutes that are undergoing suppression in *Chelydra*. They have persisted in the Emydidæ probably because they were needed to fill in the angles between the plastrals and marginals. *Aroma-*

CHRYSEMYS MARGINATA (188 specimens examined).

No	Sex.	Length in mm.	Breadth in mm.	Right Side.	Left Side.
1	F	104	80	Medium II. Medium III.	
2	F	116	88	Small II.	
3	F	102	81	Large I. Large II:	Large II.
4	M	85	67	Medium I. Medium II.	Small III.
5	F	90	73	Small II.	Small II.
6	M	96	72	Large I.	
7	F	98	80		Large I.
8	?	55	50	Medium I. Medium II.	Large II.
9	M	72	58	Small II.	Small II.
10	F	113	86	Large II.	Large II. (See Fig. 43)

A tabulation of the above results shows:

Graphemys:

Right Side.		*Left Side*
I.	4 scutes	5 scutes
II.	20 "	20 "
III.	13 "	10 "
Total:	37 "	35 "

Chrysemys:

Right Side.		*Left Side.*
I.	4 scutes	2 scutes
II.	8 "	4 "
III.	1 "	1 "
Total:	13 "	7 "

chelys odorata (Fig. 53) shows a curious survival of inframarginals, having invariably only two scutes, one large and the other very small and vestigial. From its position, separating the pectoral shields from the marginals, I would homologize this large scute with no. II. and the vestigial scute with no. I. Complete suppression of the inframarginal row has occurred in the terrestrial genera of the Emydidæ.

2. *Interplastrals.*

The occurrence of traces of the interplastral row are not nearly so frequently found as those of the inframarginals. Yet they are sufficiently numerous and definite to note in this connection. Traces have been found in *Chelydra*, *Graptemys* and *Chrysemys*. In preparing a list of these occurrences it will be convenient to number the places where such scutes might occur,

A, *B*, *C*, *D* and *E*, beginning at the anterior end. Two specimens of *Chrysemys marginata* have extra scutes at *A* (Fig. 47). Two specimens of *Chelydra* have extra scutes at *C* (Fig. 45). One specimen of *Graptemys* has extra scute at *D* (Fig. 48). One specimen of *Graptemys* has a pair of extra scutes at *E* (Fig. 46). The primitive condition was probably one in which a scute was present at each point of union of four plastron scutes, but the fact that even in the tail of *Chelydra* this row is either partially or wholly wanting indicates the rather uncertain character of the row. In the specimens listed above scutes are found occurring in four places out of a possible five. No doubt a larger collection would serve to fill in this gap.

I consider these recurrences as true reversions to ancestral conditions; and that they come under the head of systematic atavism I see no reason to doubt.

How the typical number of scute rows seen in our modern tortoises has been acquired has, perhaps, been sufficiently discussed and it now seems necessary to consider the processes that have brought about the reduction of the number of scutes in a row — for it is beyond dispute that such a reduction has taken place.

3. *Supernumerary Scutes in a Row on the Carapace.*

The literature on this subject is limited to one paper, Gadow's much-discussed "Orthogenetic Variation in the Scutes of Chelonia," that was published in Willey's Zoölogical Results in 1899. The author gives a very interesting account of the conditions found in the common loggerhead turtle, *Thalassochelys caretta*. He has gathered together a miscellaneous assortment of some sixty-nine specimens of various sizes, principally newborn, from many parts of the world. On the basis of this collection he comes to the conclusion that scute reduction proceeds along certain definite lines. His observations, however, are limited to reductions in the neural and costal rows. According to Gadow, the ideal ancestral condition is one in which the neural and costal bony plates determine the number of scutes. The author's idea is that there was originally a scute for each of these plates.

Starting with this ideal condition as stage I., he finds the nearest approach to it in specimen 1, that has 8 left costals of which 2 are vestigial, 8 right costals of which 1 is vestigial, and 8 neurals. The greatest reduction is that seen in specimen 26, which has 5 left costals of which 1 is vestigial, 4 right costals, and 7 neurals of which 1 is vestigial. This latter specimen is reduced below the normal for the species, which is arbitrarily said to possess 6 neurals and 5 pairs of costals. This condition is said to be the goal toward which every young *Thalassochelys caretta* is striving.

The following stages are mapped out in diagram, following Gadow, to show the sequence in scute reduction in the chelonian carapace:

Stage I. — Hypothetical, eight neurals and eight pairs of costals. Neurals and costals lie in the same transverse plane and coincide with neurals and costal plates.

Stage II. — Eight neurals and eight pairs of costals, the latter fitting with their inner angles dovetailed between two successive neurals. Rearrangement probably brought about by the partial reduction of one pair of costal scutes. This reduced pair is probably the second.

Stage III. — Eight neurals and seven pairs of costals, the original second costals suppressed, original third becoming second, etc.

Stage IV. — Seven neurals and seven pairs of costals, but fifth neural and fourth pair of costals (original fifth), in a state of reduction.

Stage V. — Six neurals and six pairs of costals, owing to complete suppression of fifth neural and fourth (original fifth) pair of costals.

Stage VI. — Six neurals and five pairs of costals, brought about by fusion of last two pairs of costals into one or, perhaps, by suppression of one pair. This is the normal condition in *Thalassochelys*.

Stage VII. — Six neurals and four pairs of costals. Normal condition in the majority of tortoises to-day, brought about by suppression of first pair of costals.

Stage VIII. — Six neurals and four pairs of costals, first neural (nuchal) greatly reduced.

Stage IX.— Five neurals and four pairs of costals, first neural (nuchal) suppressed as seen in pleuroderous tortoises.

Beyond this last stage chelonians have not ventured yet, at least normally.

The order of loss in scutes is according to Gadow: (1) No. 2 costals, (2) no. 7 neural, (3) no. 5 neural and no. 4 (original no. 5) costals, (4) no. 7 or 8 costals (by fusion or suppression), (5) no. 1 costals, (6) no. 1 neural.

Gadow's paper, while most suggestive, must be criticised in several particulars, but before proceeding to the criticism it will be necessary for me to produce the data that to a large extent form the basis of the criticism. The data are derived from a collection of a large number of abnormal specimens, principally of two species, *Graptemys geographica* and *Chrysemys marginata*. Gadow worked on a species that is normally abnormal — if such an expression be permissible. He selected the commonest condition and arbitrarily called it normal. As a matter of fact, there is no normal or fixed condition. The species *Thalassochelys caretta* is evidently in a highly variable state as to scute number and arrangement, and no stability has as yet been attained. The species I have studied have, on the contrary, reached an advanced state of stability. Yet a sufficiently large number of abnormalities occur to give one nearly as many examples as Gadow had. Out of 476 specimens of *Graptemys*, varying from embryos to adults and taken at random, there occurred 48 specimens with supernumerary carapace scutes, while 188 *Chrysemys* yielded 8 such abnormal specimens. Four other species belonging to widely diverse groups yielded one abnormality apiece. It seems probable that abnormalities of exactly the kind that I have found so plentifully in the case of *Graptemys* and *Chrysemys* are to be found in any species if enough specimens be examined.

In order to economize space in the tabulation of these abnormalities brevity in the nomenclature of these vestigial scutes must be attained by numbering them. Combining Gadow's figures with my own results, I have good reason to believe that vestigial scutes occur between every two surviving normal scutes and that the first, second and last costals are also found in a vestigial condition. On this basis, then, there were eleven neurals and ten

pairs of costals. These, if numbered from anterior to posterior, would give the numbers 1, 3, 5, 7, 9 and 11, to surviving neurals, and numbers 2, 4, 6, 8 and 10 to vestigial or lost neurals; the numbers 3, 5, 7 and 9 to surviving costals, and numbers 1, 2, 4, 6, 8 and 10 to vestigial or lost costals. In the tabulation these numbers will be used without further explanation. Furthermore, the sex, length and breadth of carapace, brief descriptions of both scutes and bony plates, will be given in separate columns. The significance of the tabulation of conditions of bony plates will be seen later when the subject of correlatior between scute and plate abnormalities is discussed. The specimens are numbered and arranged in the order of abnormality, the specimens with largest number of extra scutes coming first, and those with less than the normal number of scutes last. Extra neurals will be listed before extra costals and the latter before extra marginals.

Two kinds of abnormality may be distinguished: symmetrical and asymmetrical. The former are less common and are important in that they furnish clearer cases and thus throw light on the latter. Under the head of symmetrical abnormalities may be mentioned extra neurals in the median line or nearly so; extra costals in pairs symmetrically placed; extra paired marginals. The great majority of abnormalities are asymmetrical, consisting of: extra neurals crowded to one side or the other but usually showing clearly enough the position they would normally occupy; unpaired costals or marginals. In the case of asymmetrical neurals it is sometimes difficult to distinguish the supernumerary scute from the normal scute, on account of the large size of the former and the fact that crowding has forced the two scutes to lie approximately side by side. There are usually correlated points of asymmetry that may be of assistance in deciding the point, but occasionally I have been compelled to trust to my judgment and may possibly have erred. Gadow would probably consider the type in which the normal and supernumerary scutes lie side by side as evidence of the original paired character of the neural row. Were it not for transitional conditions this view might be tenable.

Occasionally it becomes difficult to determine which of five costals is the supernumerary scute, but a reference to the mar-

GRAPTEMYS GEOGRAPHICA.

No.	Fig.	Sex.	Length in mm.	Breadth in mm.	Scute Abnormalities	Plate Abnormalities.
1	20	?	60	54	Complete transverse row of 6 large scutes in middle region. Difficult to diagnose. Double 6 neural (large). L 6 costal (large). R 4 costal (large). Paired marginals.	Plates not fully formed.
2	6	F	188	144	Median 2 neural (large). Paired 1 costals (large). Extra R marginal (large).	No. 1 procaudal fused with 8 neural. Extra R marginal.
3	35	?	44	42	R 8 and 10 neurals (large). R 10 costal (medium). L 10 costal (small).	Plates not fully formed.
4	4	F	122	97	3 neural partially divided. Probably indicates fusion of 3 and 4 neural. Paired 1 costals (large). R marginal (large).	R Marginal.
5	5	F	200	145	R 8 and 10 neurals (large). R 8 costals (large).	9 neural. Double extra procaudal. R costal (medium). L costal (small).
6	3	F	189	139	R 10 neural (large). R 10 costal (large). R marginal (medium).	9 neural. Double extra procaudal. Paired 9 costals (large). R marginal.
7	13	M	98	71	R 8 and 10 neurals (large). R 8 costal (large).	Normal.
8	14	M	84	70	L 10 neural (large). 9 neural, partly divided, and probably represents 8 and 9 neurals fused. L 10 costal (medium).	Normal.
9	9	F	98	81	L 8 neural (large). 10 and 11 neurals fused. L costal (medium).	Normal.
10	36	?	52	47	R 8 and 10 neurals (large). Lacks a R marginal.	Bones not formed.
11	34	(embryo)	21	18	L 8 and 10 neurals (large).	Bones not formed.
12	37	"	25	21	R 10 neural (large). R 10 costal (medium).	Bones not formed.
13	8	F	174	136	R 10 neural (large). R 10 costal (large).	Extra procaudal.
14	10	M	113	76	L 10 neural (medium). L 10 costal (medium).	Normal.
15	12	M	98	70	R 10 neural (medium). R 10 costal (medium).	9 neural. Paired 9 costals.

GRAPTEMYS GEOGRAPHICA.—*Continued.*

No.	Fig.	Sex.	Length in mm.	Breadth in mm.	Scute Abnormalities.	Plate Abnormalities.
16	40	?	57	51	L 10 neural (medium). L 10 costal (medium).	Bones not formed.
17	Same as 10	M	87	73	L 10 neural (medium). L 10 costal (medium).	Normal.
18	7	F	170	138	6 and 7 neurals partially fused. 9 and 11 neurals completely fused. L 6 costal (medium).	Only photographic record retained.
19	56	(embryo)	12	10	R 8 neural (large). R marginal.	Ribs very abnormal.
20	17	M	89	69	Paired 10 costals (small).	Normal.
21	See 17	F	160	118	Paired 10 costals (medium).	Normal.
22	See 17	?	61	54	Paired 10 costals (medium).	Bones not formed.
23	See 17	F	209	169	Paired 10 costals (medium).	Normal.
24	26	?	51	45	Paired 1 costal (medium).	Bones not formed.
25	38	(embryo)	23	19	Median 8 neural (large).	Bones not formed.
26	39	(embryo)	19	16	L 8 neural (large).	Bones not formed.
27	23	F	192	154	Median 10 neural (medium).	Normal.
28	15	M	83	67	L 10 neural (medium).	Normal.
29	11	M	109	82	L 6 neural (large).	Normal.
30	22	M	75	63	Paired extra marginals (small).	Paired extra marginals (small).
31	16	M	78	67	R 10 costal (large).	Normal.
32	See 16	M	82	70	R 10 costal (large).	Normal.
33	See 16	F	72	62	R 10 costal (medium).	Bones not formed.
34	See 16	?	52	47	R 10 costal (medium).	Bones not formed.
35	See 16	?	63	56	R 10 costal (medium).	Bones not formed.
36	21	F	66	57	L 10 costal (medium).	Bones not formed.
37	See 21	?	57	51	L 10 costal (medium).	Bones not formed.
38	See 21	?	29	26	L 10 costal (medium).	Bones not formed.
39	See 21	?	60	54	L 10 costal (medium).	Bones not formed.
40	See 21	F	73	63	L 10 costal (medium).	Bones not formed.
41	See 21	F	99	82	L 10 costal (small).	Normal.
42	See 27	?	47	42	L marginal lacking.	Bones not formed.
43	27	?	58	52	L marginal lacking.	Bones not formed.
44	19	F	72	64	R marginal lacking.	R marginal lacking.

GRAPTEMYS GEOGRAPHICA.— *Concluded.*

No.	Fig.	Sex.	Length in mm.	Breadth in mm.	Scute Abnormalities.	Plate Abnormalities
45	See 19	?	58	53	R marginal lacking.	Bones not formed.
46	18	F	77	65	Paired marginals lacking.	Paired marginals lacking.
47		(embryo)			Paired marginals lacking and costals not fully differentiated.	Bones not formed.

CHRYSEMYS MARGINATA.

No	Fig.	Sex.	Length in mm.	Breadth in mm.	Scute Abnormalities.	Plate Abnormalities
48	31	F	130	93	R 8 and 10 costals (large). R 10 costal (large).	Extra procaudals.
49	32	F	73	61	L 8 and 10 neurals (large).	Normal.
50	30	M	104	80	Paired 10 costals (large).	Normal.
51	25	?	52	47	Paired 4 costals (medium).	Bones not fully formed.
52	24	?	57	50	L 4 or R 6 neural.	Bones not fully formed.
53	29	F	84	67	R 10 neural (large).	Normal.
54	28	M	97	66	L 1 costal (medium).	Normal.
55	41	F	73	63	L 8 costal (medium).	Normal.

ginals will usually settle the point, as the normal condition has a very characteristic arrangement of these two sets of elements.

Another source of difficulty arises from the complete or incomplete fusion of adjacent scutes. Fusion is due to the inhibition of the process of division into epidermal areas at a rather late embryonic stage. In some cases the fused scutes show their separate identity, after a year or two of growth, by a separation of their growth rings. Coker has called attention to several such cases in connection with *Malaclemmys centrata* and I have observed the same phenomenon in the marginals of *Graptemys* on several occasions. Usually, however, the indications are clear enough to enable one to recognize the individual elements in a fused scute. It seems reasonable in the present discussion to consider the number of scute primordia involved in a fusion and to give them the full rank of independent scutes.

As in the previous tabulation, the arbitrary terms, large, small and medium, are used. L or R in connection with neurals will

indicate that the extra scute is crowded to left or right. The same letters indicate the side on which extra costals and marginals occur.

The following isolated abnormalities have come to hand and may be listed :

Large specimen of *Terrapene carolina* has a R 10 costal (medium).

Medium-sized shell of *Cyclemys dentata* has paired 6 costals (medium). See Fig. 42.

Two medium-sized specimens of *Chelydra serpentina* have L 10 costal (large).

Large *Aromochelys odorata* has R 10 costal (medium).

A reference to the literature enables me to list a considerable number of similiar abnormalities. The names used in the references will be retained.

1. *Ptychemys elegans*, Agassiz, L., Contributions to the Natural History of the U. S., Plate I, Fig. 13, showing: L 4 neural, paired 4 costals, paired extra marginals. (Figured in this paper as Fig. 33.)

2. *Chelopas insculptus*, Parker, G. H., paired marginals lacking.

3. Same : R 8 and 10 neurals, R 10 costal, R marginals lacking.

In Catalog of Shield Reptiles in the British Museum.

4. *Emys vermiculata* (Tab. XIII.), 24 neural, R 8 and 10 neural.

5. *Emys singuinulenta* (Tab. XV.), R 6 neural, R 6 costal, L 4, 6 and 10 costals.

6. *Cyclemys dentata* (Tab. XIX.), median 4 neural, R 6 neural, L 8 neural.

7. *Chelodina oblonga* (Tab. XXIV.), R 8 neural.

In Historia Testudinum, Schoepff, J. B., 1792.

8. *Testudo cinera* (Tab. III.), Fig. 2, paired 1 costals.

9. *Testudo areolata* (Thunberg), Tab. XXIII., median 8 neural, L 6 costal.

10. *Testudo planiceps*, XXVII., L 4 costal.

Discussion.

A scute, whether normal or supernumerary, is a separate and definite entity, resulting from a definite embryonic primordium.

The fact that supernumerary scutes have been found between all of the normal scutes as well as at both ends of the costal series must have some significance. If one assumes that these supernumerary scutes represent the atavistic recurrence of scutes that have been lost in the course of phylogeny, it is possible that the following tabulation will throw some light on the sequence of loss.

Neurals.		*Costals.*	
No. of Scute.	Numbers of Recurrences.	No of Scute.	Numbers of Recurrences.
2	1	1	6
4	2 (one doubtful)	2	0
6	5 (one doubtful)	4	3
8	11 (one doubtful)	6	4 (one doubtful)
10	16 (one doubtful)	8	4 (one doubtful)
		10	35

It will be seen that the most frequent recurrences are at the posterior end of the carapace, and that, with the exception of the first costal, the frequency of recurrence diminishes as we proceed anteriorly. What significance attaches to this fact? It seems quite probable that the most frequent recurrences represent the most recent losses and the rarest recurrences the most ancient losses. This rule held good for the suppression of rows of scutes and should apply here also.

On this basis then we can at least say that the succession of suppression was in general antero-posterior, that the earliest losses occurred at the anterior end of the carapace and the most recent losses at the posterior end. One might go further and say that in the neural series the order of suppression was probably 2, 4, 6, 8 and 10. The antero-posterior order of loss is not so clear in the case of the costals, as no. 1 costal recurs more frequently than any other except no. 10. This means a modification of the regular mode of progression. In the costal series it is probable that the antero-posterior succession of losses was interfered with by the rounding-in of the marginals both anteriorly and posteriorly. This rounding-in would necessarily begin about medially and proceed in two directions, hence the second supernumerary scute would be put under pressure before the first and the eighth before the tenth. The antero-posterior

tendency, however, would bring about the suppression of anterior scutes, as a whole, before posterior scutes.

Evidences are not wanting that scutes may be suppressed and the method of suppression seems clear. In a specimen of *Cyclemys dentata*, listed as no. 58 and figured on Plate III., Fig. 42, the paired sixth costals are being encroached upon by the seventh costals. The anterior growing margins of the latter have pushed in under the posterior edges of the former in such a way as to severely cut into their growth centers. The dotted line shows the amount of encroachment. Several specimens of *Graptemys* show the same phenomena, and the scutes encroached upon are always the supernumerary ones. This may be looked upon as a recurrence of an ancestral condition and we may infer that the loss of certain scutes has been brought about through the encroachment, more and more severe with succeeding generations, of more vigorous upon less vigorous scutes, resulting in the final complete suppression of the latter. We must also suppose that the rudiments of the lost scutes lie dormant in the embryonic tissues and occasionally for some reason reappear more or less completely. Those that have been suppressed for the longest time would naturally reappear least often and *vice versa*. On this basis, then, we may safely say that the order of loss is orthogenetic if by this we simply mean onward development.

Applying the same methods to Gadow's figures I find a very general agreement, although I am unable to agree with the author's interpretations. The vestigial scutes that occur in Gadow's figures are: neurals 2, 8 and 10; costals 1, 2, 4, 6, 8 and 10. No. 2 costal was not found in my specimens, but is so clearly seen in Gadow's Fig. 1 that I have introduced it into my system. It is possible that No. 2 costal was the most ancient loss and hardly likely to recur in specialized types such as *Graptemys* and *Chrysemys*, since it occurred only once in Gadow's specimens of *Thalassochelys*. It will be seen that Gadow finds no vestiges of neurals 4 and 6. An examination of his figures will show that *Thalassochelys* has attained a high degree of fixity in the anterior portions of the mid-neural series, while all other regions are still in a decidedly variable condition. Hence we are

unlikely to find vestigial scutes in this region unless a much larger number of specimens is examined.

Carapace abnormalities have been pictured by authors for over a century and I have on my lists fourteen species, belonging to widely diverse groups, that show the same general abnormalities.

These scattering cases could scarcely be used in determining the order of loss of scutes, but are of importance in that they show that certain abnormalities that are comparatively rare in *Graptemys* and *Chrysemys* occur with a fair degree of frequency in other forms. For example: neurals 4 and 6, and costals 1, 4 and 6 occur from 2 to 4 times in these specimens. The prevalence of abnormalities of this sort over such a wide range of forms strengthens my idea of the universality of the process of scute reduction in *Chelonia*. I have no doubt that such abnormalities will be found in any species if enough forms are examined.

In Gadow's diagrams illustrating the progressive reduction of epidermal scutes (p. 217) it will be seen that the order of reduction differs from the one I have proposed in two points; in the first place he indicates that no. 10 neural is suppressed before no. 8, but this is not borne out by his own figures. Figs. 4, 6, 7, 14, 20, 26, show no. 10 persisting after the total suppression of no. 8, Fig. 26 being especially convincing. Figs. 8, 9, 10, on the other hand, show no. 8 persisting after the suppression of no. 10. The balance is decidedly in favor of the earlier suppression of no. 8, yet there must have been some individual variation in this matter. My own figures show that no. 8 recurs twelve times as compared with seventeen times for no. 10. In my own specimens there are eight cases in which nos. 8 and 10 neurals recur together, nine cases of no. 10 recurring alone, and only four of no. 8 recurring alone. It would seem then that these two scutes were undergoing a process of suppression at about the same time, but that no. 8 was in most cases the first to disappear.

In the second place it seems clear that no. 1 costal persisted longer than no. 10 in *Thalassochelys*, but that the opposite was the case in all the forms in my collection can scarcely be doubted. no. 10 recurs thirty-six times and in many species, while no. 1 recurs only six times.

Some rather remarkable conclusions are expressed in Gadow's paper and should be discussed in this place.

1. He makes the following statement: "Abnormalities are 4 to 7 times as common in new-born as in mature specimens, hence scute reduction must take place during the lifetime of the individual." I have not had the opportunity of putting this matter to a test in the case of *Thalassochelys*, but the examination of several complete nests of *Graptemys* has brought to light the following facts. Two nests containing respectively thirteen and fourteen embryos showed no abnormalities. One nest containing fourteen just-hatched young showed one slight abnormality, a vestigial no. 10 costal. A fourth nest in which twelve eggs came to maturity contained five decidedly abnormal specimens, listed as nos. 11, 19, 25, 26 and 48. This means that barely 10 per cent. of the embryos of four broods are abnormal, while out of 476 specimens of *Graptemys* 48 were abnormal in the carapace scutes, a little over 10 per cent. A large proportion of Gadow's new-born specimens came from one nest, the whole brood of which was abnormal. The others were taken in small sets from various collections, and I believe that such specimens had been preserved because of their abnormalities. A survey of my tabulations will show that abnormalities are no more common in one size than in another. Finally, Coker, in a very recent preliminary paper, delivered before the American Society of Zoölogists in Philadelphia, December, 1904, claims that observations on embryos of *Thalassochelys* gave no support to the theory of Gadow.

2. Gadow considers that certain specimens (Figs. 6 and 24) show evidences that the neural row was originally a double one. That this was the case seems very unlikely from an examination of such primitive conditions as are seen in the tail of *Chelydra* and in the neural keel of *Dermochelys*. The appearances seen in Figs. 6 and 24 may be due to the crowding of linear members of the row until they come to lie side by side. Indications of an approximation to this condition are not uncommon in the specimens which I have had to deal with.

4. *Supernumerary Scutes on the Plastron.*

The *plastron* has, as a rule, reached a higher degree of fixity in the matter of numbers and arrangement of scutes than has the carapace, but that this portion of the chelonian armor has not always had so fixed a character may be seen in the high state of variability of *Aromochelys odorata*, which is almost as marked as that seen on the carapace of *Thalassochelys*. In *Aromochelys* the number of plastron scutes varies from 14 to 9 and all intermediate conditions are readily found. Fig. 52 shows the largest number of scutes seen in the specimens of my collection. In this case there is a well-developed extra pair of scutes between the abdominals and femorals. Fig. 53 shows the commonest condition in which there are the usual five pairs of plates and the gulars are partially fused. Fig. 54 shows the extreme of reduction in which the pectorals have been lost either through crowding or fusion, and the gulars have fused into a single median element.

As in the case of the carapace, we find in several species that have attained a high degree of fixity in the plastron, marked traces of supernumerary scutes. Fig. 49 shows the plastron of a small specimen of *Chelydra* that has an extra pair of scutes between femorals and anals. Fig. 50 shows another specimen of *Chelydra* with a vestigial scute on the right side between humeral and abdominal. Fig. 51 shows a specimen of *Chrysemys* with a well-marked supernumerary scute on the left side between abdominal and femoral. In Figs. 49, 50, 51 and 52 we have supernumerary scutes in four places out of a possible five. As yet I have been unable to find supernumerary scutes between gulars and humerals.

Losses seem to have taken place in two ways: by fusion and by crowding out. Some curious examples of the latter might be mentioned. In *Chelydra* the abdominals have been forced to the sides, but have been retained to bridge the gap between the small plastron and the margin of the much larger carapace. In other cases the pectorals have played a similar rôle. Van Lidth de Jeude describes a specimen of *Testudo ephippium* (Gthr.), in which the pectorals have been crowded to the two sides like the abdominals of *Chelydra*. Other specimens of the same species, according to Rothschild, have the same abnormality to a greater

or less degree. The Catalogue of Shield Reptiles in the British Museum shows a specimen of *Monouria fusca* (Tab. III.), in which the pectorals are crowded to the margin of the plastron and have become small and triangular. The same volume shows a specimen of *Sternotherus Derbianus* (Tab. XXII.), in which the pectorals seem to have a tendency to be suppressed or crowded to one side.

On the whole it seems evident that an orderly suppression of alternate scutes has taken place in the plastron as well as in the carapace.

5. *Correlated Abnormalities in the Scutes and Bony Plates.*

The next question that comes up for discussion is whether or not there is any correlation between scutes and bony plates. It has long been noticed by morphologists that there is a certain definiteness about the relative positions and sizes of scutes and plates. This may be described in brief as a definite overlapping of bony sutures by scutes. In the marginal series (see Fig. 1) this is seen in its simplest form — every bony suture being covered by a scute. In the neural and costal series one scute as a rule covers one whole plate and half of two adjoining plates. This arrangement is modified in the anterior and posterior regions. In the former the nuchal plate is partially overlapped by six scutes, viz.: nuchal, first pair of marginals, first pair of costals (normally involving only small corners of the plate), and the first neural. The first costal scutes cover the first and half of the second costal plates as well as the inner edges of first, second, third and fourth marginal plates. The last neural covers normally parts of eight plates, viz.: the two procaudals and the anterior margin of the pygal, about half of the eighth neural and eighth pair of costals, and the anterior margins of the eleventh marginals. Only in the middle portions of the carapace is any definiteness of arrangement seen, yet there is a marked fixity of relations even in the most specialized regions. Gadow bases his reduction series upon an arbitrary connection between these structures, according to which there was originally a scute for each vertebra and rib. He gives no reason for assuming a vital connection between these structures, but simply implies one. In an earlier portion of the

present paper it has been shown that there is no ontogenetic connection between the scutes and plates, the former being laid down before the latter have begun to form, while the latter appear comparatively late in development as mere outgrowths of the ribs and neural spines.

If, however, there be any essential connection between these scutes and plates, we would expect to find scute irregularities and abnormalities associated with plate irregularities and abnormalties and *vice versa.*

G. H. Parker ('99) expresses himself at some length on this point in a paper entitled "Correlated Abnormalities in the Scutes and Bony Plates of the Sculptured Tortoise." He describes in detail two abnormal specimens and on this slender basis reaches some rather general conclusions.

Specimen no. 1 has extra eighth and tenth neurals and a small right tenth costal. No plate abnormalities are found in the neurals or costals, but one right marginal plate and a corresponding scute are lacking. Parker designates these conditions as: (*a*) Scute abnormalities unassociated with plate abnormalities, (*b*) scute abnormalities associated with plate abnormalities.

Specimen no. 2 has normal neural and costal scutes, but lacks an entire horizontal row of plates consisting of a neural, a pair of costals and a pair of marginals. The lack of marginal plates is associated with the lack of a corresponding pair of scutes. These conditions are designated as: (*a*) Plate abnormalities unassociated with scute abnormalities, and (*b*) scute abnormalities correlated with plate abnormalities. Parker finds the second specimen shorter than the average normal specimen of the same sex in the proportion of 1.298 to 1.313. This is due to the loss of a vertebra and pair of ribs.

In both cases it will be seen that the only real correlation occurs in the marginals and that here the correlation is perfect. Another correlation not mentioned by the author is seen in the second specimen, where there is a reduction of marginals in sympathy with the reduction in neurals and costals. This correlation is, I believe, of a different sort from that seen in the marginal plates and scutes, in that a common cause has brought out the same general effect in both sets of structures. In both cases of

correlation in the marginals Parker concludes that the abnormality is in the anterior portion of the carapace, but an examination of his figures fails to convince me that he has any criterion for thus locating the point of suppression or recurrence of scutes, for a loss anywhere in the marginals would necessitate a general readjustment with reference to the costals. Yet the author concludes that correlated abnormalities are likely to occur only in the anterior portion of the carapace.

To aid this hypothesis he adds a second one, based on a paper by Harrison ('98), in which it is shown that in the frog larva the ectoderm is proliferated chiefly at the anterior end and the mesoderm chiefly at the posterior end. This would cause the ectoderm to slide back over the mesoderm. Parker concludes that the ectodermal structures in the carapace have migrated away from their mesodermal connections so that the posterior scutes are far from their original plates, while in the anterior part of the carapace the scutes are over the same plates that they originally covered.

This ingenious hypothesis loses its force when, after the examination of large numbers of abnormalities, it is found that nearly all true correlations of plates and scutes occur at the posterior end of the carapace.

A reference to the tabulation of abnormalities will bring to light the following facts:

1. Specimens 2, 4, 6, 30, 45, 47, 61 and 62 show very precise correlation of abnormalities in the marginals. All abnormalities in the marginal scutes are correlated with similar abnormalities in the plates.

2. Specimens 5, 6 and 15 have abnormalities of both plates and scutes of the costal series, but in no case are these strictly correlated. In all three specimens the extra costal plates are paired while the extra costal scutes occur on the right side only. These specimens are abnormally long and the undue length may be the common cause of both extra plates and scutes.

3. No. 9 neural plate recurs in the same three specimens (5, 6 and 15) and is associated in each case with one or more extra neural scutes (Nos. 8 or 10). Examination shows that these extra neural plates are irregular structures, are not associated with the neural processes of vertebræ, and hence are to be con-

sidered in the same category as the procaudals. On this account I am strongly of the opinion that they have had a dermal origin like the procaudals.

4. Specimens 5, 6, 9, 13 and 49 have extra procaudal, either single or double, and in each case this plate abnormality is associated with the recurrence of an extra scute in the posterior part of the neural row. The procaudals are certainly of dermal origin and were probably the bony cores of the last neural scutes before the crowding-out process began. Consequently the reappearance of an extra plate and extra scute in this region may with justice be considered as a case of correlation.

5. Specimens 7, 8, 14, 17, 20, 21, 23, 27, 29, 31, 33, 41, 50, 51 and 58 have various kinds of scute abnormalities and perfectly normal plates.

Summarizing, we find that the only invariable correlations are in the marginals which represent scutes with their bony supports (the plates) only slightly separated from one another; that correlations between extra procaudals (also true dermal plates) occur in five cases and no case of plate abnormality in this region is without a scute abnormality; that correlations between an extra neural of small size and irregular shape occur in every case; that extra paired costal plates are associated with asymmetrical extra costal scutes; and that very frequently scute abnormalities appear without any corresponding plate abnormality.

It would appear then that abnormalities are never truly correlated except in regions where dermal plates persist and never in the anterior part of the carapace, because the plates of that region, except the nuchal, have given way to the periosteal plates of the neural and costal series. The nuchal plate still possesses its scute in a reduced condition, so we would not expect to find any abnormality in that region.

Only in the regions of dermal plates do we find any interdependence of plates and scutes, because only here, according to our theory, is there any genetic connection between dermal and epidermal structures.

That we find no true correlations between the plates and scutes of the neural and costal series is just what we would expect from our knowledge of their ontogenetic independence. In the most irregular specimens, having the most grotesque scute displace-

ments and additions in the mid-neural and costal regions, there is not a single case of sympathetic plate abnormality.

Another strong case in favor of the entire independence of these structures is furnished by examples of vertebral distortions. A paper by Wandolleck (1904) entitled "A Hump-Backed Tortoise," describes a specimen of *Testudo Græca* in which the neural and costal plates were in utter confusion, due to lateral curvature of the spine. Yet the number and arrangement of the scutes was perfectly normal. Another case in point is that of a deformed embryo in my possession that has ten ribs on one side and the number of costal scutes is perfectly normal (Fig. 56).

Further evidence for the independence of neural and costal scutes and plates is derived from the fact that certain land tortoises undergo striking modifications of plates in order to form a dome-shaped shell of great strength. The costal plates become, dovetailed one into the other and are decidedly wedge-shaped. This condition is not followed in any respect by the scutes, which retain their typical form and arrangement. A. Bienz ('94) describes this condition in *Dermatemys mavii*, and shows that the form and the arrangement of the plates conform with the most approved architectural principles.

The origin of the bony plates of the plastron has been thoroughly worked out and in each case a plate is to be interpreted as a modification of preëxisting bony structures — clavicle, sternum or abdominal ribs. If such structures as these have been transformed into large, flat, bony plates without any fusion of fascia bones or any outside factors of any sort, why is it necessary to explain the costal and neural plates as other than mere modifications of the preëxisting ribs and neural spines?

6. *Correlation in Scute Abnormalities.*

Two tendencies may be noted in this connection:

1. Scutes of the same horizontal row have a tendency to recur together (see Figs. 3, 5, 6, 7, 8, 9, 10, 12, 13, 14, 20, 31, 33 35, 40). A 10 neural most frequently is accompanied by a 10 costal, etc.

2. In asymmetrical abnormalities the tendency is for supernumerary neurals, costals and marginals to occur on the same side (see Figs. 3, 5, 7, 8, 9, 10, 12, 13, 14, 31, 35, 40).

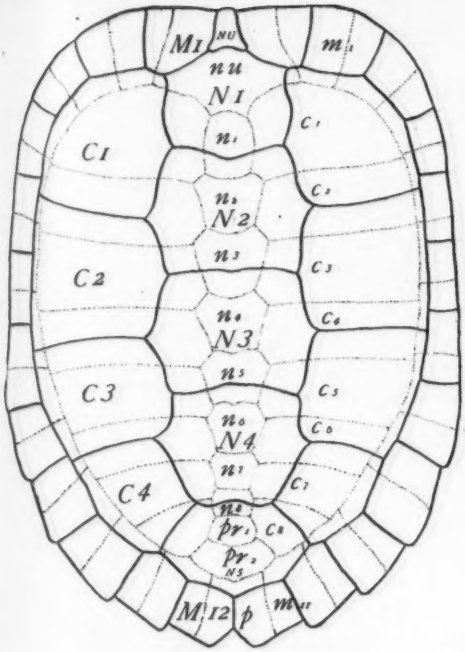


Fig. 1

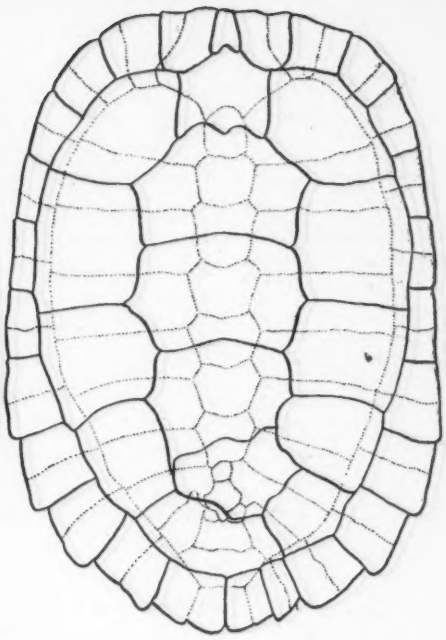


Fig. 3

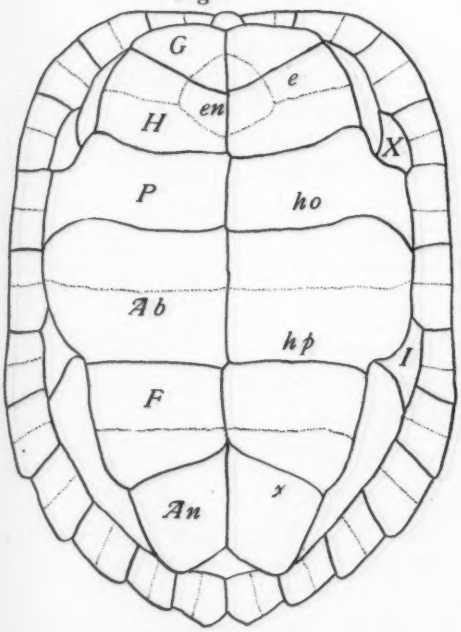


Fig. 2

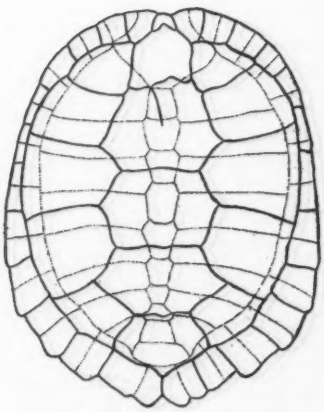


Fig. 4

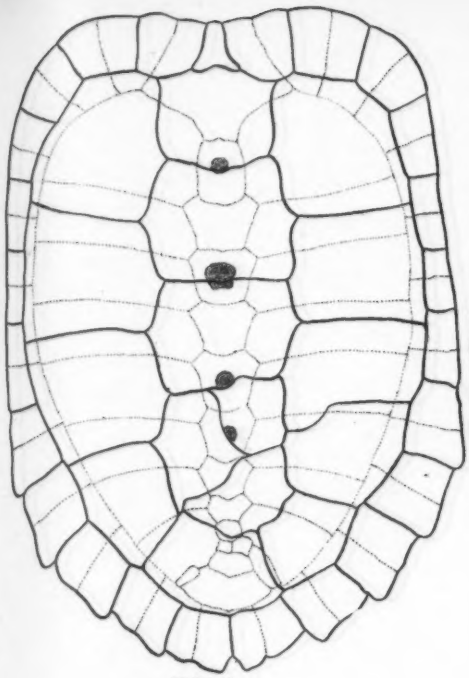
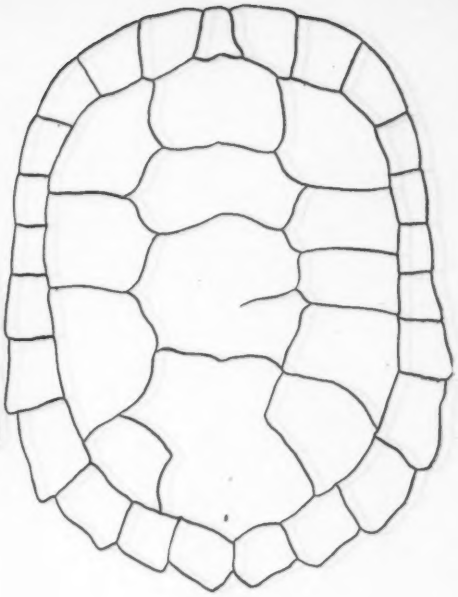


Fig. 5



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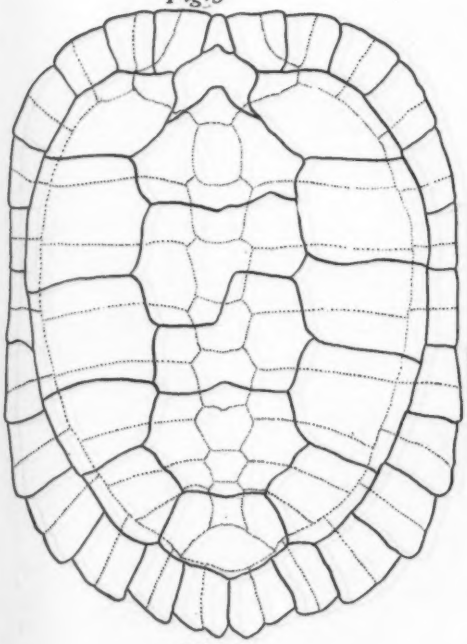

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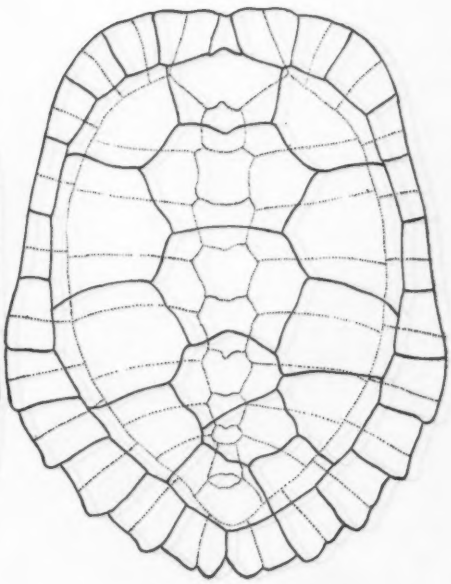

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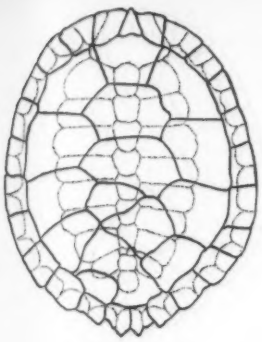

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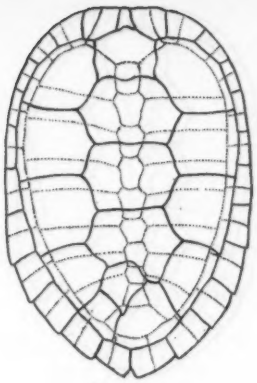

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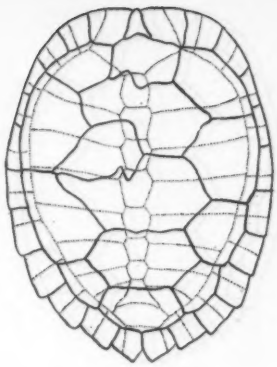

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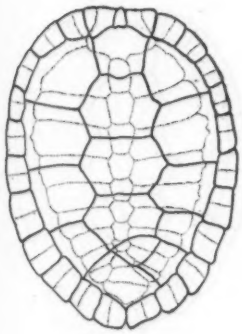

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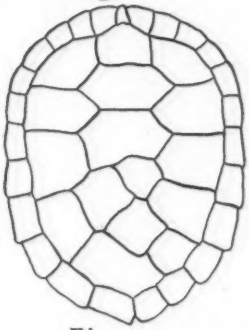

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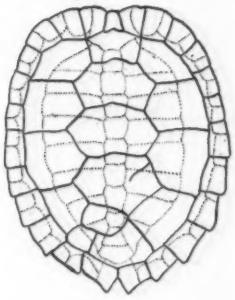

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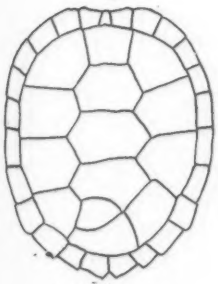

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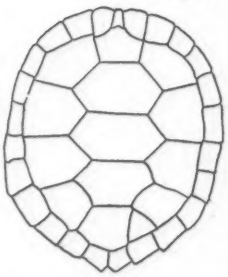

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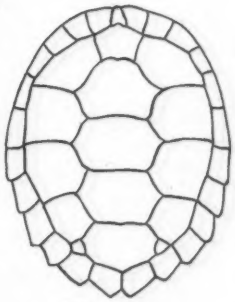

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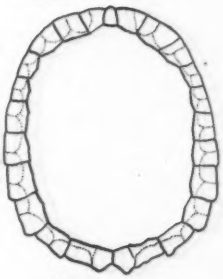

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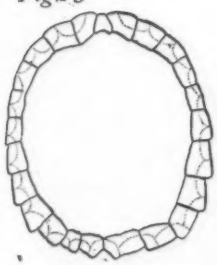

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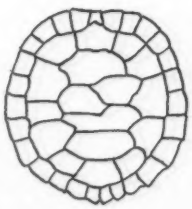

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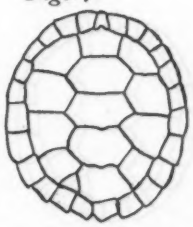

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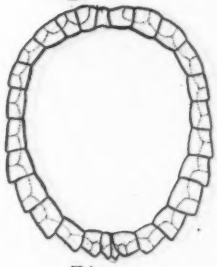

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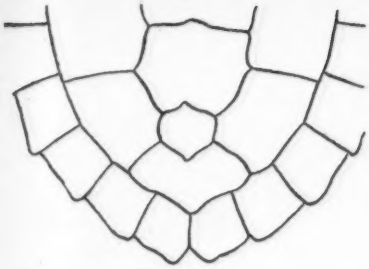

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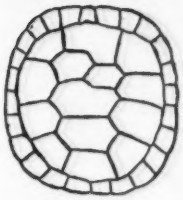

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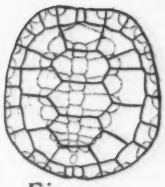
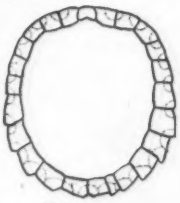


Fig. 25

Fig. 26



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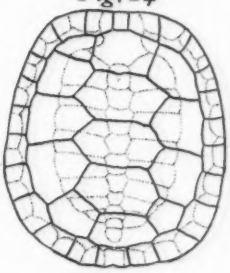

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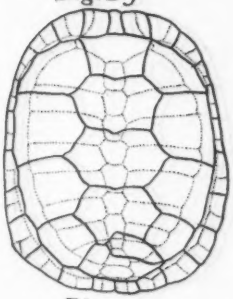

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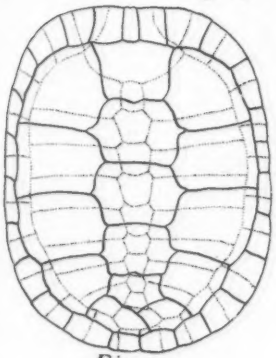

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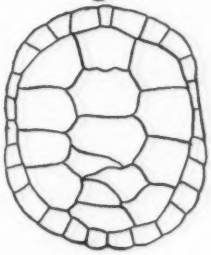

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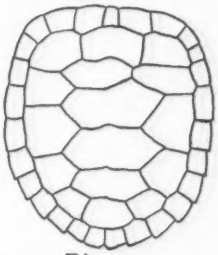

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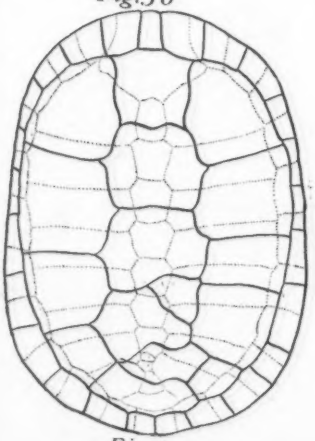

Fig. 31

Fig. 34

Fig. 35

Fig. 36

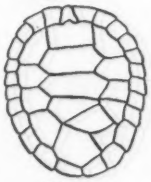

Fig. 37

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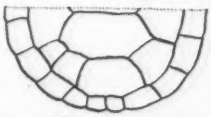
Fig. 39

Fig. 40



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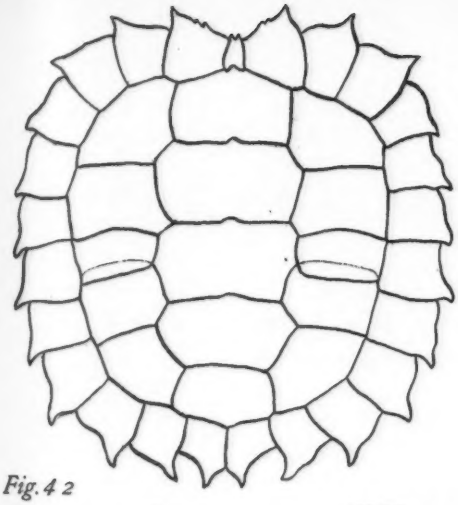

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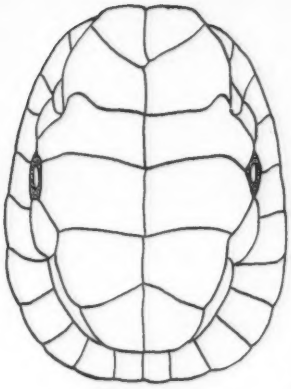


Fig. 43

Fig. 49

Fig. 50

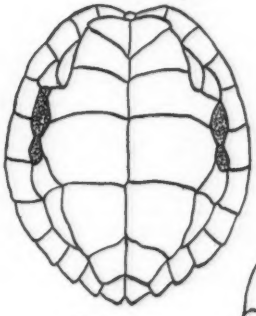
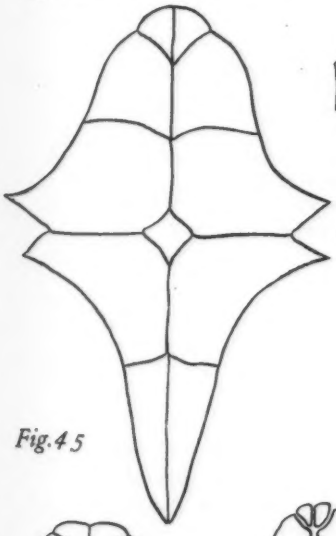


Fig. 44

Fig. 46



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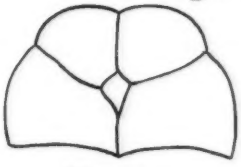

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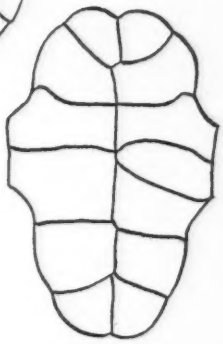

Fig. 51

Fig. 48

Fig. 52

Fig. 53

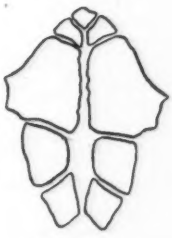

Fig. 54